

## MCP-1 secretion in lung from nonsmoking patients with coal worker's pneumoconiosis

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**ABSTRACT:** Exposure to coal dust leads to the development of coal worker's pneumoconiosis (CWP), a disease associated with an accumulation of macrophages in the lower respiratory tract. Mechanisms controlling monocyte recruitment are still poorly understood. Since monocyte chemoattractant protein-1 (MCP-1) is recognized as a potent chemotactic factor for blood monocytes, we analysed the presence of MCP-1 in the pulmonary compartment of patients with CWP.

Bronchoalveolar lavage fluid (BALF) from 16 nonsmoking control subjects and 27 nonsmoking CWP patients (16 with simple pneumoconiosis (SP) and 11 with progressive massive fibrosis (PMF)) was analysed. Alveolar macrophages (AMs) were purified by adherence and BALF was concentrated tenfold by lyophilization. MCP-1 was measured in BALF and in 3 h AM supernatants using a sandwich enzyme-linked immunosorbent assay (ELISA). The localization of MCP-1 in lung tissue was determined by immunohistochemistry on tissue sections from three patients with CWP and two control subjects.

MCP-1 levels were significantly higher in concentrated BALF from patients with SP or PMF (median 370 and 555 pg·mL<sup>-1</sup>, respectively) than in those from control subjects (median 11 pg·mL<sup>-1</sup>) ( $p < 0.001$ ). Released MCP-1 in AM supernatants was enhanced in patients with CWP (median 83 pg·mL<sup>-1</sup>) but compared to controls (median 41 pg·mL<sup>-1</sup>) this level did not reach significance. Although significantly increased, AM counts in BALF from patients with CWP did not correlate with MCP-1 levels. MCP-1 levels in BALF correlated with MCP-1 levels in AM supernatants ( $\rho = 0.47$ ;  $p < 0.02$ ). In control lung specimens, MCP-1 was expressed by a few AMs, type II pneumocytes and perivascular smooth muscle cells. CWP sections were characterized by an increased number of AMs and mainly by the presence of fibroblasts (in the myogenic area of fibrotic lesions) and hyperplastic type II pneumocytes, which were strongly immunostained for MCP-1.

Our data demonstrate that: 1) patients with coal worker's pneumoconiosis have a marked pulmonary overproduction of monocyte chemoattractant protein-1; and 2) in addition to alveolar macrophages, fibroblasts (probably myofibroblasts) and hyperplastic type II pneumocytes may also be responsible for this increased level of monocyte chemoattractant protein-1 in coal worker's pneumoconiosis.

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Exposure to coal dust leads to the development of coal worker's pneumoconiosis (CWP) [1, 2]. CWP is defined by the presence of coal dust-induced lesions distributed throughout the lung, and is usually divided into two categories according to abnormalities on chest radiography: simple pneumoconiosis (SP), in which opacities are smaller than 1 cm, and progressive massive fibrosis (PMF), in which opacities are more extensive [3]. CWP is characterized by pulmonary fibrosis, emphysema and macrophage infiltrates [4, 5]. Although the pathophysiological mechanisms of CWP remain incompletely understood, current concepts suggest that alveolar macrophages (AMs) play a central role in the pathophysiology of this disease: AMs from pneumoconiotic patients are known to spontaneously produce several mediators, including cytokines, such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), potentially implicated in the induction of the inflamma-

tory response and in the development of a subsequent extensive fibrosis [6]. AMs are predominantly derived from differentiated peripheral blood monocytes and, to a limited extent, from the local replication of these cells [7, 8].

Recruitment of blood monocytes occurs in response to a gradient of chemotactic factor(s) produced by the resident cells. However, the mediators involved in this process have not been evaluated in CWP. One of the most potent chemotactic factors for blood monocytes is the monocyte chemoattractant protein-1 (MCP-1), a 76 amino acid peptide that belongs to the chemokine family (which includes the factor regulated upon activation in normal T-cells, expressed and secreted (RANTES), macrophage inflammatory protein (MIP), interleukin (IL)-8 and others) [9]. MCP-1 is produced by a variety of immune and inflammatory cells, upon activation by

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mediators such as TNF- $\alpha$  [10–15]. MCP-1 can also activate monocytes, increasing their cytostatic activity against several tumour cell lines, the generation of respiratory burst, the release of lysosomal enzymes and proinflammatory cytokines (IL-1, IL-6), and the expression of monocyte adhesion molecules (CD11c/CD18 and CD11b/CD18), which play a key role in migration [16, 17]. Taken together, these observations suggest that MCP-1 may be involved in the development of the chronic macrophagic inflammation observed in CWP.

In the present study, we analysed the presence of MCP-1 in bronchoalveolar lavage fluid (BALF) from patients with SP or PMF and from control subjects. We also determined MCP-1 concentration in 3 h AM supernatants and the localization of this chemokine in lung tissue by immunohistochemistry (IHC).

### Material and methods

#### Patients

Twenty seven nonsmoking coal workers from the "Bassin Houillier du Nord et du Pas-de-Calais" (France) were studied. The diagnosis of CWP and the separation into two groups (SP and PMF) were made in accordance with criteria described previously [3]. Sixteen patients had SP, their ages ranged 55–73 yrs (mean $\pm$ SEM 63 $\pm$ 2 yrs) and their mean duration of underground dust exposure was 25.9 $\pm$ 2.4 yrs (range 15–32 yrs). Eleven patients had PMF, their ages ranged 60–80 years (mean 69 $\pm$ 2 yrs) and their mean duration of underground dust exposure was 26.0 $\pm$ 1.7 yrs (range 20–34 yrs). Healthy subjects (n=16), never exposed to coal mine dust, were selected as controls; their ages ranged 24–72 yrs (mean 52 $\pm$ 4 yrs). All subjects were lifelong nonsmokers or they had quit smoking for more than 5 yrs. None of the patients or controls were treated with corticosteroids at the time of bronchoalveolar lavage (BAL).

#### Bronchoalveolar lavage

Oral informed consent was obtained for BAL from both pneumoconiotic patients and healthy subjects. The study protocol was approved by the hospital's Ethics Committee. BAL was performed, as described previously, by instillation of saline buffer into the bronchoalveolar tree under fiberoptic bronchoscopy [4]. Cells were obtained by centrifugation, as described below. BALF was concentrated tenfold by lyophilization. Cellular characteristics of the BAL are summarized in table 1.

#### Cell isolation and alveolar macrophage culture

Macrophages were purified from BAL: the lavage fluid was filtered through sterile surgical gauze and centrifuged

at 400 $\times$ g for 10 min at 4°C. After two washings, the pellet was resuspended at a cell concentration of 1.5 $\times$ 10<sup>6</sup> cells·mL<sup>-1</sup> in RPMI 1640, containing 5% heat inactivated foetal calf serum (FCS; Gibco Laboratories, Grand Island, NY, USA) and 200  $\mu$ g·mL<sup>-1</sup> of sodic mezlocilline (Bayer, Germany). Cell viability was assessed by trypan blue exclusion and was at least 95% in all cases. Differential cell counts were performed using eosin/methylene blue (RAL, Paris, France) stained cytocentrifuge preparations of recovered cells. Cells were allowed to adhere to plastic Petri dishes (2 mL in 35 mm diameter well; Becton Dickinson, Franklin Lakes, NJ, USA) for 2 h at 37°C, 5% CO<sub>2</sub>. The nonadherent cells were removed by three washings with RPMI medium. Adherent cells contained more than 95% AMs. AM supernatants were collected after 3 h culture, filtered through 0.22  $\mu$ m Millipore filters (Molsheim, France), and frozen in aliquots at -20°C until quantification of MCP-1.

#### Sandwich enzyme-linked immunosorbent assay (ELISA) for MCP-1

A sandwich ELISA for MCP-1 was developed with two polyclonal antibodies. This ELISA could quantitatively detect MCP-1 ranging 50–10,000 pg·mL<sup>-1</sup>. To assess specificity, several structurally related cytokines, which may be contained in the biological sources, were tested for cross-reactivity. None of these control cytokines (IL- $\beta$ , IL-2, IL-4, IL-6, IL-8, IL-10, interferon- $\gamma$  (IFN- $\gamma$ ), MIP-1 $\alpha$ , RANTES and TNF- $\alpha$ ) was detected. This ELISA showed the advantage of using commercial antibodies and of needing very small volumes of biological sample to obtain reproducible measures. Microtitre plates (Nunc, Denmark) were coated with 50  $\mu$ L·well<sup>-1</sup> of rabbit polyclonal anti-MCP-1 antibody (Genzyme, Cambridge, MA, USA) (4  $\mu$ g·mL<sup>-1</sup> 0.1 mM NaHCO<sub>3</sub>, pH 8.2) overnight at 4°C. The plates were then washed twice with phosphate-buffered saline (PBS)/0.5% Tween (wash-buffer) and nonspecific binding sites were blocked with PBS, 3% bovine serum albumin (BSA) for 30 min at 37°C. Plates were rinsed once with wash-buffer. Duplicates either of MCP-1 standards (Pepro Tech Inc., Rocky Hill, NJ) or various dilutions of test samples (50  $\mu$ L·well<sup>-1</sup>) were then added.

After 90 min at 37°C, plates were washed four times and 50  $\mu$ L·well<sup>-1</sup> of 3  $\mu$ g·mL<sup>-1</sup> goat polyclonal anti-MCP-1 antibody (RD System, Abingdon, UK) in PBS with 1% BSA was added. After 90 min at 37°C, plates were washed four times and each well received 50  $\mu$ L of rabbit biotinylated anti-goat immunoglobulin G (IgG) antibody (Sigma, St Louis, MO, USA; 1/500 in PBS/1% BSA) for 90 min at 37°C. After four washings, 50  $\mu$ L·well<sup>-1</sup> of avidin-peroxidase (CLB, Amsterdam; 1/10,000 in Tris buffered saline (TBS)/Tween) was added for 30

Table 1. – Characteristics of bronchoalveolar lavage from the study population

Group	n	BAL volume mL	Total cell count $\times 10^4$ cells·mL <sup>-1</sup>	Alveolar macrophages		Lymphocytes		Neutrophils	
				%	$\times 10^4$ cells·mL <sup>-1</sup>	%	$\times 10^4$ cells·mL <sup>-1</sup>	%	$\times 10^4$ cells·mL <sup>-1</sup>
Controls	16	127.9 $\pm$ 7.4	11.9 $\pm$ 1.4	83 $\pm$ 2	9.9 $\pm$ 1.3	14 $\pm$ 2	1.7 $\pm$ 0.3	3 $\pm$ 1	0.3 $\pm$ 0.1
SP	16	105.4 $\pm$ 11.0	24.7 $\pm$ 6.4*	86 $\pm$ 2	21.8 $\pm$ 5.8*	11 $\pm$ 2	1.8 $\pm$ 0.3	2 $\pm$ 1	0.9 $\pm$ 0.7
PMF	11	114.1 $\pm$ 11.0	33.8 $\pm$ 6.1***	87 $\pm$ 2	30.2 $\pm$ 5.9***	9 $\pm$ 2	2.6 $\pm$ 0.4	2 $\pm$ 1	1.0 $\pm$ 0.3

All values are expressed as mean $\pm$ SEM. SP: simple pneumoconiosis; PMF: progressive massive fibrosis; BAL: bronchoalveolar lavage. \*: p<0.05; \*\*\*: p<0.001, significantly different from controls.

min at room temperature. The plates were washed five times with PBS/Tween and twice in TBS before the addition of 50  $\mu\text{L}\cdot\text{well}^{-1}$  of substrate solution. The reaction was stopped by addition of 50  $\mu\text{L}\cdot\text{well}^{-1}$  HCl 4 N and the absorbance was read at 492 nm on an ELISA reader. MCP-1 concentrations were calculated by interpolation from the standard curve. A value of 1  $\text{pg}\cdot\text{mL}^{-1}$  was given to samples below the sensitivity threshold. A compilation of 13 separate standard curves is presented in figure 1.

#### Immunohistochemistry on lung tissue

Lung tissue fragments were obtained by lobectomy performed either on patients presenting a lung cancer but never exposed to coal mine dust ( $n=2$ ), or on pneumoconiotic patients with lung cancer ( $n=3$ ; with SP and with PMF). Lung specimens were taken in the subpleural region, as far as possible from the neoplastic area, fixed with PBS/4% paraformaldehyde and embedded in paraffin for serial sectioning (6–7  $\mu\text{m}$ ). Rehydrated lung sections were permeabilized by digestion for 10 min at 37°C with type II trypsin (Sigma), washed in PBS and saturated for 1 h with PBS/4% human serum (HS). Sections were then overlaid over night at 4°C with antihuman MCP-1 polyclonal antibody (Genzyme, 6  $\mu\text{g}\cdot\text{mL}^{-1}$ ) previously immunoadsorbed with sepharose-coated with normal human serum. Dilutions were performed in PBS/4% HS. After several washes with PBS, sections marked with the antihuman MCP-1 polyclonal antibody were incubated for 90 min with biotin-conjugated goat anti-rabbit IgG antibody (Sigma; 1/300), washed, incubated for 30 min with extravidin-alkaline phosphatase (Sigma; 1/200) and washed again. Colour development was obtained with a fast-red solution (Sigma). Gill's haematoxylin was used as counterstain. Immunostaining was completely inhibited by the addition of anti-MCP-1 antibody preincubated with recombinant human MCP-1, which demonstrated antibody specificity.

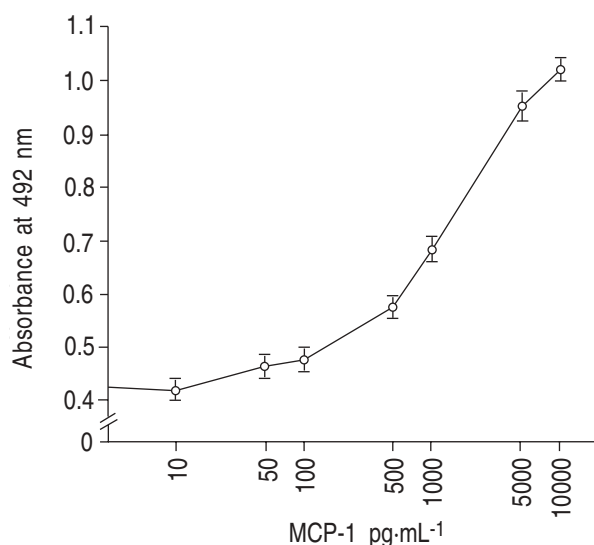


Fig. 1. — Standard curve of the sandwich enzyme-linked immunosorbent assay (ELISA) for MCP-1. Each point represents the mean of 13 separate determinations carried out in duplicate and vertical bars indicate standard error. MCP-1: monocyte chemoattractant protein-1.

#### Statistical analysis

Data are expressed as median and interquartile range (IQR). Differences between groups were tested for statistical significance by using the Kruskal-Wallis test and the Mann-Whitney U-test. A p-value less than 0.05 was considered significant. Correlation coefficient was computed by using the Spearman test.

## Results

#### Cellular analysis of BAL

The volume of lavage fluid recovered was similar for pneumoconiotic patients and control subjects. The total cell count in BAL from patients with SP or PMF was significantly higher than that from control subjects (table 1). The percentage of AMs, lymphocytes and neutrophils did not differ between pneumoconiotic patients and control subjects. The total number of lymphocytes and neutrophils per millilitre of recovered fluid was similar in BAL from patients with CWP and from control subjects. In contrast, the total number of AMs per millilitre of recovered fluid was significantly increased in BAL from patients with SP or PMF as compared with control subjects. There was no significant difference between SP and PMF.

#### MCP-1 in lavage fluids

MCP-1 was undetectable or at a very low level in concentrated BALF from control subjects (median 11  $\text{pg}\cdot\text{mL}^{-1}$ ; IQR 50  $\text{pg}\cdot\text{mL}^{-1}$ ;  $n=14$ ). The MCP-1 level was significantly higher in concentrated BALF from patients with CWP (405  $\text{pg}\cdot\text{mL}^{-1}$ ; IQR 1,030  $\text{pg}\cdot\text{mL}^{-1}$ ;  $n=20$ ) than in those from controls ( $p<0.001$ ) (fig. 2). The difference between SP (370  $\text{pg}\cdot\text{mL}^{-1}$ ; IQR 610  $\text{pg}\cdot\text{mL}^{-1}$ ;  $n=10$ ) and PMF (555  $\text{pg}\cdot\text{mL}^{-1}$ ; IQR 1,060  $\text{pg}\cdot\text{mL}^{-1}$ ;  $n=10$ ) was not significant. There was no correlation between the number of AM and the MCP-1 level in BALF.

#### MCP-1 in AM supernatants

There was a trend towards an increased level of MCP-1 in AM supernatants from patients with CWP (83  $\text{pg}\cdot\text{mL}^{-1}$ ; IQR 192  $\text{pg}\cdot\text{mL}^{-1}$ ;  $n=24$ ) compared with control subjects (41  $\text{pg}\cdot\text{mL}^{-1}$ ; IQR 81  $\text{pg}\cdot\text{mL}^{-1}$ ;  $n=11$ ), but the difference was not significant (fig. 3). Indeed, there was a large overlap of MCP-1 levels in AM supernatants from SP (85  $\text{pg}\cdot\text{mL}^{-1}$ ; range 1–1,350  $\text{pg}\cdot\text{mL}^{-1}$ ;  $n=15$ ) and PMF (78  $\text{pg}\cdot\text{mL}^{-1}$ ; range 1–4,150  $\text{pg}\cdot\text{mL}^{-1}$ ;  $n=9$ ). The normal range was defined as the 95th percentile value of the control data (91  $\text{pg}\cdot\text{mL}^{-1}$ ). The MCP-1 level in AM supernatants was higher than the upper limit in 7 out of 15 cases of SP (47%) and in 4 out of 9 cases of PMF (44%). The number of AMs in BAL did not correlate with the MCP-1 level in AM supernatants. However, there was a correlation between MCP-1 levels in AM supernatants and MCP-1 levels in BALF ( $\rho=0.47$ ;  $p<0.02$ ).

#### Immunohistochemical localization of MCP-1 protein in lung tissue section

Lung sections from patients with CWP showed aggregations of coal dust-laden macrophages beneath the

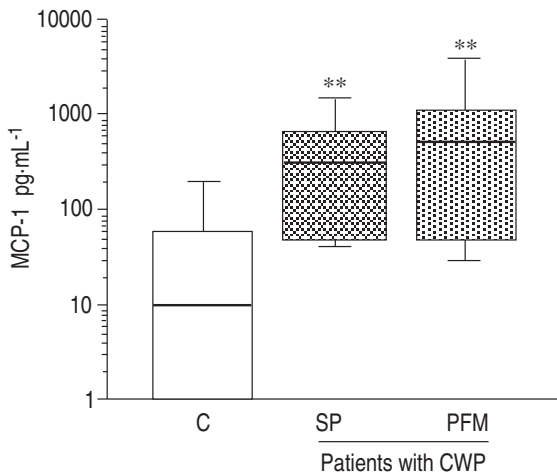


Fig. 2. — Profile of MCP-1 secretion in BALF concentrated, ten-fold, from control subjects (C; n=14) and from patients with simple pneumoconiosis (SP; n=10) or progressive massive fibrosis (PMF; n=9). Data are presented as box plots. Horizontal lines represent the median, squares the 25th and 75th percentiles, and bars the 10th and 90th percentiles. \*\*:  $p < 0.01$ , significantly different from the control. MCP-1: monocyte chemoattractant protein-1; CWP: coal worker's pneumoconiosis.

pleural surface, in alveolar spaces and in fibrous tissues, presenting as spider-shaped micronodules. Polarized light microscopy revealed birefringent particles scattered throughout the nodules. Myofibroblastic cells in the micronodules were not associated with vessels or bronchi. The lung sections from patients with CWP were also characterized by the hyperplasia of many type II pneumocytes.

In tissue sections from control subjects, a few AMs were positive for MCP-1 expression by immunostaining with anti-MCP-1 antibodies (fig. 4A). In addition, in some areas, some type II pneumocytes and perivascular smooth muscle cells were positive for MCP-1.

In lung sections from patients with CWP, perivascular smooth muscle cells were found to express MCP-1

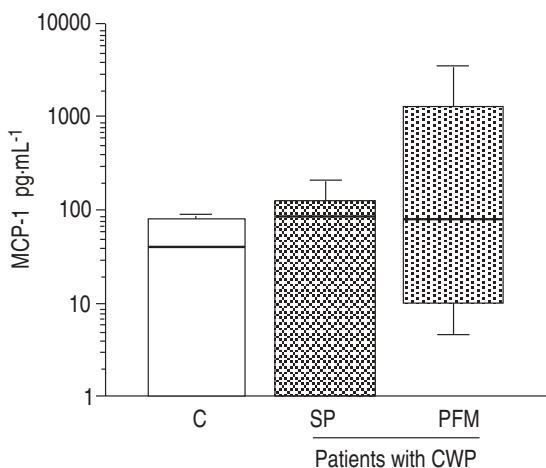


Fig. 3. — Profile of MCP-1 secretion by alveolar macrophages (AMs) from control subjects (C; n=11), and from patients with simple pneumoconiosis (SP; n=15) or progressive massive fibrosis (PMF; n=9). Supernatants were obtained from a 3 h culture of  $1.5 \times 10^6$  AMs·mL<sup>-1</sup>. Data are presented as box plots. Horizontal lines represent the median, squares the 25th and 75th percentiles, and bars the 10th and 90th percentiles. No statistical difference was observed between any groups for this parameter. MCP-1: monocyte chemoattractant protein-1; CWP: coal worker's pneumoconiosis.

in the same way as in control sections. The proportion of AMs positive for MCP-1 was similar for the two controls and two patients with CWP (range 21–28% for the four subjects), and higher for one CWP patient (47%). Interstitial macrophages were positive. Fibroblasts in the fibrotic areas were marked; their aspect as well as their location suggested that they were myofibroblasts. The hyperplastic type II pneumocytes strongly expressed MCP-1 (fig. 4B and C). Immunostaining with the control nonimmune serum showed negative results.

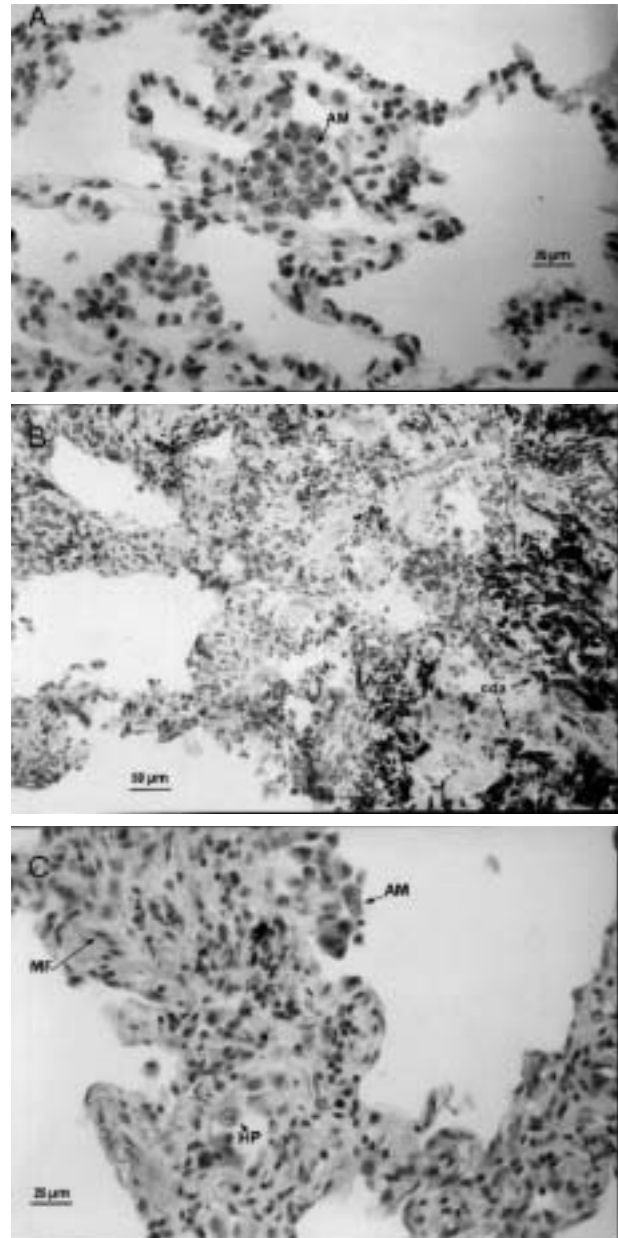


Fig. 4. — Immunohistochemical localization of MCP-1 protein in lung tissue sections. A) Representative lung section from control subject immunostained with antibody directed against MCP-1. Alveolar macrophages (AMs) were positive for MCP-1 expression. B) Representative section of lung tissue from a patient with CWP, immunostained with antibody directed against MCP-1. Coal dust accumulation (cda) and fibrotic areas (fa) are visible. C) Representative section of lung tissue from patient with CWP, immunostained with antibody directed against MCP-1. AMs, hyperplastic type II pneumocytes (HPs) and myofibroblasts (MFs) were positive for MCP-1 expression. MCP-1: monocyte chemoattractant protein-1; CWP: coal worker's pneumoconiosis.

## Discussion

CWP is associated with a dramatic increase in the number of macrophages in alveolar spaces, due mainly to an influx of peripheral blood monocyte influx. This macrophagic accumulation results from a local production of monocyte chemoattractants, but the producing cells and signals that attract the inflammatory cells into the pulmonary tissue are still poorly documented. In this study, it was shown that the MCP-1 level was significantly elevated in BALF from patients with CWP as compared with controls. The MCP-1 concentration in BALF was closely correlated with the MCP-1 level in AM supernatant. Immunohistochemistry of lung sections indicated that other cell types, mainly perivascular smooth muscle cells, type II pneumocytes and fibroblasts also expressed MCP-1.

The MCP-1 levels in BALF from controls were heterogeneous (range 1–330 pg·mL<sup>-1</sup>), suggesting that MCP-1 may be induced by everyday exposure to ambient dust and pollution, or that an interindividual difference in MCP-1 gene regulation could exist. However, the MCP-1 level was significantly higher in BALF from patients with CWP than from controls, suggesting that this chemokine might be implicated in the development and maintenance of the macrophage alveolitis associated with CWP. However, since no correlation was found between the number of AMs in BALF and the MCP-1 level, it is likely that MCP-1 may not be the only factor responsible for the recruitment of AMs towards alveolar spaces. Similarly, it has been demonstrated that in BALF from patients with sarcoidosis or idiopathic pulmonary fibrosis (IPF), both MCP-1 level and monocyte chemotactic activity were increased, but without correlation between these two parameters [18]. Many other chemokines (MCP-2, MCP-3, MIP-1 $\alpha$ , RANTES) and recently IL-13, have been shown to have a chemotactic activity for monocytes [19, 20]. An *in vivo* study in the rat has demonstrated that tracheal instillation of mineral dust (SiO<sub>2</sub>, TiO<sub>2</sub>) increased MIP-1 $\alpha$  in the whole lung, indicating that this cytokine may be involved, at least in part, in the recruitment of inflammatory cells after particle deposition [21]. Although a recent study indicated that MCP-1 was the most powerful monocyte chemokine, it is likely that the combined action of these different monocyte chemoattractants might be important in lung inflammatory processes [22]. The lack of correlation between MCP-1 level and the number of AMs in BAL might also indicate that monocyte recruitment depends not only on the gradient of chemokines, but also implicates several successive steps, including adherence, rolling and extravasation, regulated by proinflammatory cytokines.

We recently demonstrated that expression of intercellular adhesion molecule-1 (ICAM-1), an adhesion molecule that plays a major role in monocyte migration, was higher in lungs from pneumoconiotic patients than in lungs from control subjects [23]. Due to its capacity to increase, *in vitro*, monocyte expression of CD11b/CD18 and CD11c/CD18 (ligands for ICAM-1) [17], MCP-1 might be involved in the adherence step during monocyte recruitment. MCP-1 also induces calcium flux, respiratory burst activity, and IL-1 and IL-6 expression in monocytes [16, 17]. Therefore MCP-1 is likely

to be a mediator for the priming of mononuclear phagocytes. Indeed, it has been shown that blood monocytes from miners, as well as peritoneal macrophages from rat exposed to aerosols of silica, *i.e.* both cell types removed from direct contact with particles, were primed for increased TNF- $\alpha$  secretion upon provocation with a second stimulus [24, 25].

Another aim of the present study was to determine which cell types express MCP-1. *In vitro* MCP-1 has been shown to be produced by several human cells types, including endothelial cells [11], fibroblasts [12], pulmonary type II pneumocytes [13], mesothelial cells [14] and blood mononuclear leucocytes [15]. We found that, in control pulmonary tissue sections, only a few AMs, type II pneumocytes and perivascular smooth muscle cells were positive for MCP-1 by IHC. In lung sections from patients with CWP, fibroblasts present in fibrotic areas were positive for MCP-1 as well as hyperplastic type II pneumocytes, which were not detected in control tissue sections. This evolution of the pattern of MCP-1 expression in CWP is different from the pattern found in IPF, where macrophages, vascular endothelial and smooth muscle cells and type II pneumocytes have been shown to express MCP-1 [26]. In CWP, we observed also a significant immunolocalization of MCP-1 in fibroblasts.

The number of MCP-1 positively immunostained AMs in CWP sections was increased, but the proportion of AMs positive for MCP-1 expression seemed to be similar in control and CWP sections. In the same way, spontaneous MCP-1 secretion by AMs was evaluated by measuring MCP-1 in AM supernatants after 3 h of culture, and no significant difference was found between patients with CWP and controls. These results are different from those concerning TNF- $\alpha$  and IL-6, since AMs from patients with CWP produced significantly greater amounts of these proinflammatory cytokines than did control AMs [27]. Thus, our results suggest that MCP-1 secretion by AMs depends on other mechanisms than those controlling the secretion of TNF- $\alpha$  and IL-6. In addition, the role of AMs in the development of the inflammatory process in CWP is more likely to be linked to the secretion of proinflammatory cytokines than of monocyte chemoattractant factors.

It was beyond the scope of the present study to determine the mechanisms responsible for MCP-1 overexpression in CWP. This overexpression is potentially the result of a direct activation by coal dust. It might also be the consequence of cell-to-cell communication, in part *via* soluble mediators, since it has been demonstrated that fibroblasts and type II pneumocytes secrete MCP-1 in the presence of activated AM conditioned media [12, 13]. Moreover, we may hypothesize a cell network activation consecutive to interaction *via* specific receptors. Indeed, it has been shown, firstly, that an increased monocyte-derived MIP-1 $\alpha$  production was induced by ICAM-1 interaction with activated endothelial cells, and, secondly that IL-8 and MCP-1 production was increased during monocyte-endothelial cell interactions, in part due to matrix protein-binding mechanisms [28, 29].

In conclusion, our data underline the fact that patients with coal worker's pneumoconiosis have a marked pulmonary overproduction of monocyte chemoattractant

protein-1. However, the lack of correlation between the number of alveolar macrophages and the level of monocyte chemoattractant protein-1 is consistent with the fact that recruitment of alveolar macrophages in coal worker's pneumoconiosis is presumably controlled by more than one mediator, including other chemokines and pro-inflammatory cytokines.

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